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THE SIGNIFICANCE OF SELENIUM AND VITAMIN E IN NUTRITION

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The history of selenium—vitamin E interrelationships

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I count it both an honour and a pleasure to be invited to come 'north of the border' to give the opening paper of this Symposium. The presentation of my paper, however, will not be easy, in view of the extreme complexity of the field. Before forming a balanced opinion about the dietary importance of selenium we must know something about the mode of action of vitamin E *per se*. This subject was already very complicated before Se came into the story, and I could indeed occupy the whole space allotted to the printed version of my paper by giving a list of references. I cannot really do more, therefore, than deal with vitamin E itself in rough outline, and so leave time to review, only a little more fully, the recent work on Se.

The discovery of vitamin E. As a convenient starting point we may go back to the famous work of Evans & Burr (1927). In their massive monograph on *The Antisterility Vitamine Fat Soluble E* they reported, as had also been found by certain other workers, that rats could not reproduce satisfactorily when given diets containing all the nutrients previously known to be required. In females the characteristic lesion was resorption of the foetuses, and in males irreversible injury to the testes. As we all know, the most familiar of the several forms of vitamin E was eventually identified as a condensation product between duroquinone, a trimethylated quinone, and phytol. It was given the name α-tocopherol, and differs from the other forms, which need not concern us today, in the number of its methyl groups, or in modifications to the phytol part of the molecule. In common with other quinone derivatives α-tocopherol acts both in vivo and in vitro as an antioxidant capable of retarding the oxidation of fats.

Se poisoning in livestock. Se came into the news, about 10 years after the discovery of vitamin E, as the cause of severe poisoning in farm animals. It had long been known that animals grazing in certain parts of the 'Great Plains' of the USA, and particularly in Nebraska, were liable to become emaciated, to lose their hair and hoofs, and to develop anaemia, cirrhosis of the liver, and skeletal erosions. Poisoning also occurred in pigs and poultry that were fed upon wheat grown in the same areas. The disease was first known as 'alkali disease', but after long research Se was

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incriminated as the toxic agent (Franke & Potter, 1935). Poisoning occurred in those regions in which the Se content of the soil, for geological reasons, was unduly high. These observations certainly conveyed no hint of Se's being beneficial, but at least they indicated that it is not biologically inert. Further support for this view came nearly 20 years later when Pinsen (1954) found that Se, together with molybdenum, was necessary to certain bacteria, typified by *Escherichia coli*, for the production of the enzyme formic dehydrogenase.

Vitamin E in non-reproductive functions. For our next advance we must return to vitamin E. Within 4 years of the publication of the Evans–Burr monograph Goettsch & Pappenheimer (1931) described a condition of 'nutritional muscular dystrophy' in guinea-pigs, which was later found to be due to deficiency of vitamin E. Ringsted (1935) reported paresis in rats kept for prolonged periods on diets deficient in vitamin E, and later Einarson & Ringsted (1938) described lesions in both the muscles and nerves of such animals. It became obvious at this point that the field of action of vitamin E was not confined to the reproductive processes. Martin & Moore (1936, 1939) described a brown coloration of the uterus in virgin rats deficient in vitamin E, and also abnormalities in the kidney. In vitamin E-deficient rats the storage of vitamin A was defective (Moore, 1940). The incisor teeth of rats lost their normal brown pigmentation (Moore, 1943). In rats given cod-liver oil, but not dosed with vitamin E, the fat depots became brown and peroxides were present (Dam & Granados, 1945). Red blood cells taken from rats deficient in vitamin E were found to be rapidly haemolysed in the presence of dialuric acid (György & Rose, 1948). In chicks vitamin E deficiency was found to cause two characteristic lesions, exudative diathesis (Dam & Glavind, 1938) and encephalomalacia (Pappenheimer & Goettsch, 1931). These could occur either simultaneously or separately.

One further lesion in the rat deserves our special attention in view of its importance in studies of Se–vitamin E interrelationships. This lesion is the massive necrosis of the liver, rapidly fatal once it starts, which was first studied by György (1947) and later with special reference to vitamin E by Lindan & Himsworth (1950) and by Schwarz (1949). Before returning to Se, however, we must deal with two further important aspects of the vitamin story.

Factors influencing vitamin E requirements. First it must be emphasized that the vitamin E requirement of any animal is not absolute and constant, but variable according to both the composition of the diet and the lesion under consideration. For example, hepatic necrosis was first produced in rats by deficiency of protein. It was soon found that this disease could be prevented either by improving the protein supply in which the limiting factor was cystine, or alternatively by dosing with vitamin E. With regard to this one lesion, therefore, the tocopherol requirement of rats given adequate amounts of protein or of cystine is nil. Schwarz (1949) spoke of hepatic necrosis as an 'ambogenous' lesion, because a combined deficiency of two nutrients was necessary for its production. Rats given ample amounts of protein are immune only from hepatic necrosis, and not from other effects of avitaminosis E.

In the opposite direction some nutrients may increase, rather than diminish, the requirement for vitamin E. The best known antagonists are polyunsaturated fatty

acids. Thus the body fat of rats given large amounts of cod-liver oil turns brown. This lesion may be prevented either by dosing with α-tocopherol, or by replacing the cod-liver oil by lard, a much less unsaturated fat. When pigmentation of the body fat is taken as our characteristic lesion, therefore, the vitamin E requirement of a rat fed upon lard appears to be nil. But again this conclusion cannot be applied to other effects of vitamin E deficiency.

Vitamin E substitutes. Secondly we must digress on the very complicated subject of vitamin E substitutes. Over 20 years ago Evans, Emerson, Emerson, Smith, Ungrade, Prickard, Austin, Hoehn, Opie & Wawzonek (1939) summarized the results of biological tests on over 100 compounds related more or less closely to α-tocopherol. Many of these substances, for example several ethers of duroquinone, were active when given in massive doses. Later much more surprising claims were made by Dam and his colleagues. From 1951 onwards they reported that numerous substances bearing no structural resemblance to α-tocopherol, but mostly having antioxidant or redox properties, could completely or partly replace the vitamin. Thus Dam, Kruse, Prange & Søndergaard (1951) found that methylene blue, thionine, thiodiphenyl-amine, tetraethyl thiuram disulphide (Antabuse), nordihydroguiaretic acid and ascorbic acid could all afford considerable, but incomplete, protection against the effects of vitamin E deficiency in chicks. Dam & Granados (1952) found that methylene blue could even prevent resorptions in rats. Moore, Sharman & Ward (1953*a,b,c*, 1954) failed to confirm this last finding. In rats, moreover, they failed to observe even partial vitamin E activity of ascorbic acid, Antabuse, or nordihydro-guiaretic acid. Methylene blue, however, was found to be completely effective in preventing some of the lesions caused by vitamin E deficiency, notably brown uterus. Certain other dyes, including malachite green and rosaniline, were also completely effective against the same range of lesions.

It appeared for a time, therefore, that artificial substitutes for vitamin E, as typified by methylene blue, could fulfil some of the functions of the vitamin but not others. Thus methylene blue, when given to rats, could completely prevent brown discoloration of the uterus and body fat, muscular dystrophy, post-mortem autolysis of the kidneys, and defective storage of vitamin A. It could not prevent resorptions, testicular degeneration, or haemolysis of the red blood cells by dialuric acid. This sharp division between the ranges of potency of α-tocopherol and its redox substitutes, however, was broken down when Singsen, Bunnell, Matterson, Koseff & Jungherr (1955) brought the antioxidant DPPD (N,N'-diphenyl-p-phenylenediamine) into the picture. The evidence at present available suggests that this substance can replace α-tocopherol in all its functions.

It may be helpful to arrange a list of some of the main lesions of vitamin E deficiency according to their resistance in responding to vitamin E substitutes, and to compare the activities of a few typical substitutes. This plan has been adopted in Table 1, in which the activities of α-tocopherol, DPPD, methylene blue, cystine and Se are compared.

Factor 3 in the prevention of liver necrosis. To understand how Se came into the story we must now return to the subject of hepatic necrosis. This disease can readily

Table 1. *Prevention of lesions in the rat by α-tocopherol, diphenyl-p-phenylenediamine,*
methylene blue, L-cystine, and sodium selenite

Lesion	α-Tocopherol	DPPD	Methylene blue	Cystine	Sodium selenite
Resorption of foetuses	+	+ ?	o	o	o
Haemolysis by dialuric acid	+	+	o	o	o
Degeneration of testes	+	+	o?	o	o
Dental depigmentation	+	+	o?	o	o
Muscular dystrophy	+	+	+	o	o?
Brown uterus	+	+	+	o	o
Brown body fat	+	+	+	o	o
Kidney autolysis *post mortem*	+	+	+	o	o
Liver necrosis	+	+	o	+ ?	+

be produced in young, rapidly growing rats by giving them a diet in which the sole source of protein is torula yeast and from which all sources of vitamin E have been removed. We may recall that Schwarz (1949) described this disease as ambogenous, because simultaneous deficiencies of two nutrients, α-tocopherol and cystine, were then thought to be necessary for its production. This statement, however, fell short of complete truth, since it was soon realized that there must be not two, but three simultaneous deficiencies for the production of hepatic necrosis. This fact was discovered through the different potencies of various types of yeast in precipitating necrosis. Thus Schwarz (1951) found that if the torula yeast in his diet was replaced by American baker's yeast hepatic necrosis did not occur. The protective power of the American yeast could not be explained by a higher cystine content. The presence of a 'Factor 3' in the American yeast had therefore to be assumed.

Schwarz, and certain other workers, began work on the isolation of Factor 3. In 1957, 6 years after the recognition of this factor, it was realized that the potency of certain concentrates resided not in their organic matter, but in their ash. Moreover when active fractions were treated with alkali, an odour suggestive of garlic was noticed. This gave a clue to the presence of Se. Schwarz & Foltz (1957) demonstrated not only that the purest Factor 3 concentrates contained Se but that inorganic sodium selenite was effective, in minute doses, in preventing hepatic necrosis in rats. Simultaneously Patterson, Milstrey & Stokstad (1957) were working on the isolation, from casein or pig tissues, of a factor which prevented exudative diathesis in chicks fed upon diets containing torula yeast. Ash from concentrates, made in the presence of alkali, was protective, but ash made in the presence of acid was inactive. These properties pointed to Se, which was indeed found to be completely effective against the exudations.

Much work has since been done on the form of combination of Se in Factor 3 concentrates and on the biological activity of numerous organic compounds containing Se. No organic compound of Se, however, appears to be more than two or three times more potent against hepatic necrosis than the same amount of Se in the form of selenite.

The biological activity of Se. The biological activity of Se is remarkable in two ways. Firstly its range of activity as judged by the number of lesions prevented, is very narrow. In the rat, as will be seen from Table 1, hepatic necrosis is prevented, but seven other characteristic lesions are not prevented. Se does not seem very effective in opposing the stress induced by the inclusion in the diet of highly unsaturated fatty acids. Moreover, I may draw attention today to the new finding (unpublished), based on work in my laboratory, that Se does not prevent post-mortem autolysis in the kidney. This lesion, perhaps, is prevented more easily than any other by most vitamin E substitutes.

Unfortunately a confident interpretation of the evidence on certain points is still impeded by disagreements between the findings of different workers. For example Irving (1959) has found that Se confers some protection against dental depigmentation, but Aterman (1959) has found it inactive against this lesion.

The second remarkable point is that Se, within its limited range of action, is effective in very minute doses. Thus Schwarz & Foltz (1957) found that as little as 4 μg Se, as sodium selenite, per 100 g diet was sufficient to prevent hepatic necrosis. This meant that Se was 500 times more active than α-tocopherol, and 250 000 times more active than L-cystine.

The discovery of the activity of Se therefore raised suspicions that the activity of many of the vitamin E substitutes might be due to the presence of this element as an impurity. Thus methylene blue contains sulphur, which can hardly be rigorously freed from Se before it is used for making the dye. We know, however, that methylene blue will prevent lesions which cannot be prevented by Se, so that in this instance our suspicions may be dismissed. Cystine and Se, however, both have about the same range of action, and the presence of 4 p.p.m. of Se in cystine could explain its activity. Schwarz, Stesney & Foltz (1959) concluded at one time that the potency associated with cystine is due to the presence of traces of Se as impurity. In contrast Yang, Dialameh & Olson (1959) could not find sufficient Se in their cystine to account for its biological activity. However, even if pure cystine is protective, most specimens must owe some of their activity to contamination with Se.

Recent developments. At this point I take comfort in the thought that I have been asked to review the history of Se–vitamin E interrelationships. History concerns the past, and it is not my duty to suggest how all the diverse items of information, which are at present available, will eventually be correlated. I may conclude my review, however, with brief mention of some recent findings, most of which suggest that there are even further complications in our story.

As a first point I may re-emphasize the apparent diversity of the roles of α-tocopherol. Rival schools of thought have regarded the vitamin either as a so-called 'non-specific antioxidant', or as a possible specific component of enzyme systems. It seems to me that the functions of α-tocopherol cannot be limited to a single metabolic effect, but must be multiple. This conclusion seems inescapable if we can be certain that the ranges of action of methylene blue and Se are different. Conflicting evidence, unfortunately, again makes a clear distinction hazardous, but methylene

blue seems to be particularly effective against fat peroxidation and muscular dystrophy, and Se particularly effective against liver necrosis and exudative diathesis. Since α-tocopherol combines the activities of both substitutes and can act in other ways as well, it would appear to exert its activity at more than one place in metabolism. A puzzling point, however, is that Se has usually been found ineffective in dealing with muscular dystrophy in experimental rats and rabbits, but has been found effective in preventing dystrophy in farm animals, as Blaxter (1962) tells us.

In support of the idea that Se may act as a biological catalyst, probably as part of an enzyme system, Gitler (1958) studied a system in which methylene blue is decolorized by cysteine. This reaction, he found, is greatly accelerated by the presence of small amounts of Se. My colleague M. G. Stanton (1962, private communication) has followed up this work, using a system in which oxygen is removed by a stream of nitrogen, and has confirmed the catalytic action of Se. Fig. 1 correlates the rates of decolorization of methylene blue by cysteine with the concentrations of selenite added. It is interesting that all the three components of this artificial system can act as vitamin E substitutes.
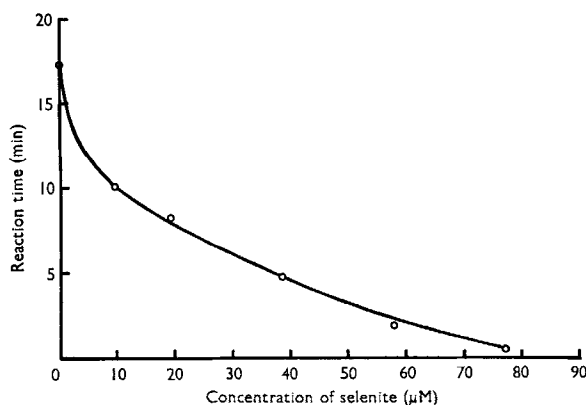

Fig. 1. Catalysis by sodium selenite of the reduction of methylene blue by cysteine. Concentration of cysteine, 16.9×10^{-3}M. Phosphate–citrate buffer, pH 6. Times are for the concentration of methylene blue to fall from $21.9 \ \mu$M to $0.94 \ \mu$M (Stanton, 1962, private communication).

Another recent finding, dealt with by Green (1962), has been that the levels of ubiquinone in the tissues may be influenced by the intake of α-tocopherol and of Se. The evidence for this view must obviously be judged on its own merits. Early experiments, both by Morton & Phillips (1959) and by myself (Moore, 1959), however, have indicated that the organs of rats severely deficient in vitamin E still contain amounts of ubiquinone within the normal range.

As I stated in my opening remarks the whole question of the mode of action of vitamin E is extremely complicated, and I must apologize if I have failed to provide the mental satisfaction of a clear and tidy picture. I must also ask pardon for sins of omission, both from my readers and from those authors whose work I have neglected. Thus I have had no time to tell about the extensive experiments by Schwarz

on respiratory decline in necrotic liver, or his claims that a 'glucose tolerance factor', containing trivalent chromium, appears to be associated with Factor 3 in its concentrates (Schwarz & Mertz, 1959). I have also had to omit the interesting work of Naftalin (1951) who found that the vulnerability of rats to hepatic necrosis was influenced by the environmental temperature. Various workers have brought sulphaguanidine (Granados, Aaes-Jørgenson & Dam, 1950), manganese (Dam & Granados, 1951), thyroid activity (McLean & Beveridge, 1952), adrenaline (Mertz & Schwarz, 1954) and cortisone (Schwarz, 1951) at least into the margin of our field.

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